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Lack of Association Between Mutation Size and Cognitive/Behavior Deficits in Fragile X Males: A Brief Report

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Previously, researchers reported molecular-neurobehavioral or molecular-cognitive associations in individuals with fra(X) (fragile X) mutation. However, not all investigators have noted molecular-behavioral relationships. Consequently, we examined prospectively 30 fra(X) males age 3–15 years from four testing sites to determine whether there was a relationship between mutation size and degree of either cognitive or adaptive behavior deficit. To measure cognitive abilities, all individuals were administered the Stanford-Binet (4th edition) IQ test. To evaluate adaptive behavior (DQ) skills, all individuals were assessed using the Vineland Adaptive Behavior Scale. To determine fra(X) status, genomic DNA from all individuals was extracted and digested with *Eco*RI and *Eag*I restriction enzymes. Southern blots were prepared and hybridized with the pE5.1 probe. The Pearson correlation coefficient between full mutation size and composite IQ score revealed a non-significant, near-zero association ($r = 0.06$; $P > .76$). The Pearson coefficient between mutation size and DQ also showed a non-significant, near-zero association ($r = 0.06$; $P > .73$). We conclude that while fra(X) mutation produces cognitive and behavior deficits in males who inherit the defective gene, there is no relationship between mutation size and degree of deficit.

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INTRODUCTION

The fra(X) (fragile X) mutation, a leading cause of inherited mental retardation (MR), results from a defective FMR-1 gene [Verkerk et al., 1991]. Unaffected carriers have a "premutation" which contains a large, unstable number of CGG repeats (50–230) in the 5′ untranslated region of the FMR-1 gene. Affected males and females, whose CGG copy number ranges upwards from several hundred to more than a thousand copies, have what is described as a "full" mutation. In addition to its unusual size, individuals with the full mutation have an abnormally methylated CpG island adjacent to the CGG repeat. The disparate number of CGG repeats between a typical premutation and full mutation, along with clinical/behavioral differences in individuals with premutations compared to those with full mutations, suggests a quantitative relationship between mutation size and degree of cognitive/behavioral deficit.

After analyzing test-retest IQ scores from 98 fully mutated males, Fisch et al. [1992] argued that there may be two types of full mutations, one which produced longitudinal declines in IQ scores, and another which produced little or no change. Staley et al. [1993] examined 57 males with mutations and noted that those with large full mutations showed no relationship between IQ scores and age, but those with small full mutations demonstrated a significant negative correlation between age and IQ. Among fra(X) females, Abrams et al. [1994] noted significant negative correlations between mutation size and IQ scores, although Taylor et al. [1994] found none. Among fra(X) males, de Vries et al. [1993] found no relationship between mutation size and IQ score. On the other hand, Rousseau et al. [1994] found that both abnormal methylation and mutation size were highly correlated with mental retardation.

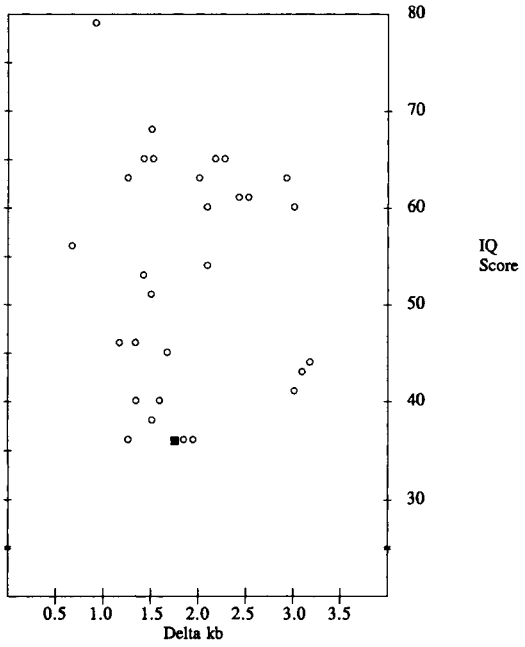

Fig. 1. Cognitive deficit (IQ score) as function of mutation size (kb) for fra(X) males (n = 30).

Therefore, the purpose of our prospective, multicenter study was to ascertain whether there was any relationship between CGG copy number in fully mutated males and their concomitant clinical/behavioral deficits.

MATERIALS AND METHODS

Subjects

From an ongoing prospective multicenter study, 30 fully mutated males between age 3–15 years were evaluated between May, 1991–October, 1994. Subjects live at home with one or both parents. Diagnosis for the mutation was made initially by cytogenetic evaluation and confirmed by direct DNA testing. Subjects were obtained and tested at each of four sites: 1) 9 were from the Chapman Institute in Tulsa, OK; 2) 8 were from the Genetics and IVF Institute in Fairfax, VA; 3) 6 were from the Ongwanada Resource Centre in Kingston, Ontario, Canada; and 4) 7 were from the Greenwood Genetics Center in Greenwood, SC.

Cognitive and Behavioral Assessment

All subjects were assessed cognitively and behaviorally. To obtain a measure of cognitive ability (IQ), a psychologist administered the Stanford-Binet test, 4th edition (SBFE) [Thorndike et al., 1986]. To obtain a measure of adaptive behavior (DQ), one or both parents were interviewed using the Vineland Adaptive Behavior Scales (VABS) [Sparrow et al., 1984]. The SBFE and VABS were administered in the same testing session.

DNA Testing

Peripheral blood from all subjects was drawn for DNA testing. Genomic DNA was extracted and digested with *Eag*I and *Eco*RI restriction enzymes, and sent to a single test center (Ongwanada), where Southern blots were prepared and hybridized with the pE5.1 probe. Photographs made from autoradiographs were returned to each testing site, where laboratory staff blind to the diagnosis evaluated and sized the bands in each lane.

Estimation of Mutation Size

Estimates of mutation size were obtained by ranking the raters' four estimates from lowest to highest and calculating the median value. A more detailed analysis of DNA testing and analysis of raters' estimates can be found elsewhere [Fisch et al., 1996].

RESULTS

To determine the relationship between mutation size and cognitive ability, composite IQ score was plotted as a function of median mutation size, and a Pearson coefficient (r) was computed. To determine the relationship between mutation size and adaptive behavior, the adaptive behavior composite (DQ) score was plotted as a function of median mutation size, and the Pearson correlation coefficient was computed. These data are presented in Figures 1 and 2. Figures 1 and 2 exhibit no particular pattern between mutation size and either IQ or DQ score. Calculation of the Pearson coefficients confirms a nonsignificant, near-zero correlation between mutation size and IQ score ($r = 0.06$; $P > .76$), and a nonsignificant, near-zero correlation between mutation size and DQ score ($r = 0.06$; $P > .73$).

DISCUSSION

In noting longitudinal differences between large full mutation and small full mutation males, Staley et al.

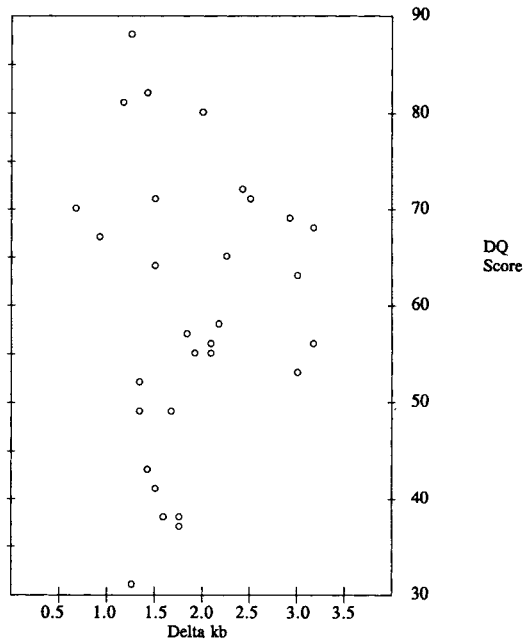

Fig. 2. Adaptive behavior deficit (DQ score) as function of mutation size (kb) for fra(X) males (n = 30).

[1993] had pooled males with large premutations and males with small full mutations into one group; hence, the disparity they observed may have resulted from within-group variation in methylation patterns rather than mutation size. McConkie-Rosell et al. [1993] noted that members from one family of males with small full mutations who were less affected were also methylated mosaics. Hagerman et al. [1994] examined the DNA of high-functioning males and observed that several had small full mutations and were methylation mosaics. More recently, Steyaert et al. [1996] reports that, in males whose mutation sizes fall in the large premutation/small full mutation range, the proportion of cells with methylated FMR-1 genes is correlated with IQ. Similarly, de Vries et al. [1996] examined activation ratios among a small sample of fra(X) females and noted similar findings. Earlier, Abrams et al. [1994] found a significant positive correlation between full-scale IQ and activation ratio in fra(X) females, comparable to the results obtained by de Vries et al. [1996]. These results suggest that proportion of methylated cells rather than mutation size is the salient factor producing cognitive deficits in fra(X) individuals.

In conclusion, fra(X) males whose mutation sizes are sufficiently large that hypermethylation of the FMR-1 gene occurs have cognitive and adaptive behavior deficits as well as other clinical manifestations of fra(X) syndrome. However, the size of the mutation in and of itself does not correlate with degree of deficit. Rather, if the size of the mutation is sufficiently large such that methylation occurs, the degree of deficit appears correlated with the proportion of cells with methylated FMR-1 genes. It suggests that, in addition to premutation or full-mutation status, proportion of methylated cells should be reported as well.

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